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Assistant Commissioner of Patents
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TRANSMITTAL OF TRANSLATION OF PRIORITY DOCUMENT

Transmitted herewith for filing in the above-referenced patent application are the following documents:

1. Verification of Translation of Priority Document of Japanese Application No. 10 (1998)-366719, filed December 24, 1998 together with a copy of the Specification, Sequence Listings, Figures, Claims and Abstract;
2. This Transmittal Letter; and
3. Return Postcard.

Applicant(s) believe that no fees are required. However, if for any reason a fee is required, the Commissioner is hereby authorized to charge any fees to Deposit Account No. 04-1105.

If the enclosed papers are considered incomplete, the mailroom and/or the Application Branch is respectfully requested to contact the undersigned at (617) 439-4444, Boston, Massachusetts.

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on February 28, 2002.

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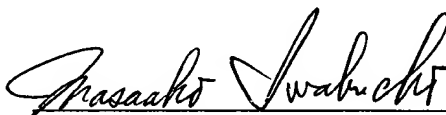
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VERIFICATION OF TRANSLATION

I, the undersigned Masaaki Iwabuchi, translator, having an office at Abe, Ikubo & Katayama, 2-8-7, Yaesu, Chuo-ku, Tokyo, Japan, declare that I am well acquainted with the Japanese and English languages, and that the attached English text is, to the best of my knowledge, a complete and accurate translation from the Japanese text of the priority document, Japanese Patent Application No. 10 (1998)-366719 (Ref. No. A98234), dated December 24, 1998.

The undersigned further declares that all statements made herein of his/her own knowledge are true, and all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like, so made, are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Date: February 13, 2002


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[Title of the Invention] UCP-2 PROMOTER AND ITS USE

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[Document] SPECIFICATION

[Title of the invention] UCP-2 Promoter and Its Use

[Claims]

[Claim 1] A DNA containing uncoupling protein-2 (UCP-2) promoter region containing the regulator sequence.

[Claim 2] A DNA described in claim 1 wherein the regulator sequence is a sequence containing peroxisome proliferator response element (PPRE).

[Claim 3] A DNA described in claim 1 wherein the regulator sequence is a sequence containing CCAAT/enhancer binding protein (C/EBP) binding sequence.

[Claim 4] A DNA described in claim 1 wherein the promoter region is a base sequence presented by position 1 to 2270 of SEQ ID NO: 1 or a base sequence containing a part of the said base sequence.

[Claim 5] A DNA described in claim 1 wherein the DNA is a recombinant vector containing a DNA containing a structural gene under control of UCP-2 promoter region containing a regulator sequence.

[Claim 6] A transformant transformed by a DNA described in claim 1.

[Claim 7] A method for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of a transformant described in claim 6.

[Claim 8] A method for screening an antiobestic drug, an antidiabetic drug, a depressor, an antihyperlipemic drug, and an antipyretic drug characterized by use of a transformant described in claim 6.

[Claim 9] A kit for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of a transformant described in claim 6.

[Claim 10] A pharmaceutical composition comprising a compound or its salt that promotes or inhibits UCP-2 promoter activity obtained using a screening method described in claim 7 or a screening kit described in claim 9.

[Detailed description of the invention]

[0001]

[Technical field to which the invention pertains]

This invention relates to a novel promoter for gene expression and its use. Specifically, this invention relates to a DNA containing the promoter region of human uncoupling protein-2 (UCP-2) gene, a transformant transformed with the said DNA, and a method for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity.

[0002]

[Prior art]

Uncoupling protein (UCP) is a proton transporter present in the mitochondrial inner membrane. Since UCP changes intracellular energy stored as fat to heat without using other energy consuming processes, UCP is considered to play an important role in maintenance of body temperature in homeothermal animals. Because of this function, UCP is considered to be an important factor that determines the efficiency of energy metabolism in homeothermal animals.

Three molecular species of uncoupling protein have been identified to date, and are called uncoupling proteins-1 (UCP-1), -2 (UCP-2 or UCPH), and -3 (UCP-3).

UCP-1, the first isolated among the uncoupling protein family, is specifically expressed in brown fat cells (Line, C.S. and Klingerberg, M. (1980), FEBS Lett., 113, 299-303; Jacobsson, A. et al. (1985), J. Biol. Chem., 260, 16250-16254; Bouillaud, F. et al. (1986), J. Biol. Chem., 261, 1487-1490). UCP-2 was isolated as a homologue of UCP-1, and confirmed to be widely expressed in various organs (Gimeno, R.E. et al. (1997), Diabetes, Vol. 46, 900-906; Fleury, C., et al. (1997), Nature Genet., Vol. 15, 269-272). UCP-3 was isolated as a UCP specifically expressed in muscle (Vidal-Puig, A. et al. (1997), Biochem. Biophys. Res.

Commun., Vol. 235, No. 1, 79-82; Boss, O. et al. (1997), FEBS Lett., 408, 33-38).

Generally, UCP-1 is considered to play an important role in maintenance of body temperature in rodents and hibernants. As for the basis, the number of brown fat cells that mainly express UCP-1 is lower in large sized animals and animal species inhabiting in relatively warm weather (Rothwell, N.J. and Stock, M.J. (1979), Nature, Vol. 281, 31-35). Thus, in these animals including human, UCP-2 or UCP-3, not UCP-1, may mainly be responsible for the control of the normal body temperature maintenance system and energy consuming process (Hosoda, K. et al. (1998), Obesity Research (Himan Kenkyu), Vol. 4, No. 3, 31-35; Enerback, S. et al. (1997), Nature, Vol. 387, 90-93).

Therefore, it may be possible to adjust the energy consumption/accumulation balance by controlling the gene expression or activity of UCP-2 or UCP-3 in these animals including human (Hosoda, K. et al. (1998), Obesity Research (Himan Kenkyu), Vol. 4, No. 3, 31-35; Enerback, S. et al. (1997), Nature, Vol. 387, 90-93). In human, enhancement of energy consumption is considered to promote consumption of not only dietary energy but also energy accumulated as fat. Accordingly, a decrease of body fat in human may lead to improvement of obesity, the major cause of lifestyle diseases which become a problem in developed countries in recent years (Fleury, C. et al. (1997), Nature Genetics, Vol. 15, 269-272).

UCP-2 is also considered to be the major cause of fever observed in immunological inflammation such as infection, and inhibition of UCP-2 gene activity may reduce fever in immunological inflammation (Shigenaga, F.R. et al. (1998), Biochim. Biophys. Res. Commun., Vol. 244, No. 1, 75-78).

In animals, especially in higher animals, organs differentiate and mature upon biogenesis, and develop to exert each function. During this process, various organ-

specific proteins are transiently or constantly expressed and provide the organ-specificity.

The general gene expression control system in animals includes the transcription induction system (promoter, enhancer). Promoter regions are generally located adjacent to the 5' upstream region of base sequences on chromosomes that are normally transcribed into messenger RNAs. Transcriptional regulator protein is bound to or dissociated from base sequence generally called regulator sequence in promoter regions, by which the transcription level of genes located downstream of the 3' region is regulated. Therefore, the transcriptional gene expression level can be estimated from the promoter activity to some extent. It is also known that the base sequences located downstream of the 3' region of a promoter do not affect the promoter activity in most cases. Therefore, promoter activity can be readily measured by substituting the transcribed messenger RNA for a base sequence encoding a protein with detectable enzyme activity (reporter). Recent technical innovation has made measurement of promoter activity using reporters very sensitive and simple, and measurement of promoter activity is used in drug screening and analysis of biological function.

For example, transcriptional regulatory factors of fat cell differentiation include peroxisome proliferation-activated receptor γ (PPAR γ) (Tontontz, P. et al. (1995), Curr. Opin. Genet. Dev., Vol. 5, 571-576), retinoid X receptor (RXR), CCAAT/enhancer binding protein (C/EBP) (Cornelius, P., et al. (1994), Annu. Rev. Nutr. Vol. 14, 99-129), etc. The transcriptional regulation by these factors are closely involved in the gene expression related to fat cells. It has been reported that the promoter regions of fat cell-related genes including UCP-2 gene contain the binding sequences for these transcriptional regulatory factors (regulator sequences). These sequences in promoters are considered to play important roles in the

actual regulation of UCP-2 transcription in vivo.

[0003]

[Problem to be solved by the invention]

Accordingly, substances that enhance expression of UCP-2 or UCP-3 gene and protein may be used as antiobestic drugs that reduce body fat content. UCP-2 is also considered to be the major cause of fever in immunological inflammation observed in infection, and substances that inhibit UCP-2 gene activity may reduce fever in immunological inflammation.

If a cell line expressing an appropriate reporter gene connected to the promoter region described above is established, the cell line may be used for screening a drug that promotes or inhibits the UCP-2 expression. In screening substances that may be used as antiobestic drugs, responses more similar to those in vivo can be obtained by including these regulator sequences in the promoter-reporter system, which is very advantageous in screening human antiobestic drugs.

However, human UCP-2 promoter containing the regulator sequence has not yet been identified, and no simple screening method using the promoter described above has been available for substances that affect the human UCP-2 gene expression.

[0004]

[Means for solving problem]

The inventors performed extensive studies, and successfully obtained the human genomic UCP-2 gene using human UCP-2 cDNA fragments as probes in attempt to establish a screening method for searching substances that affect the human UCP-2 gene expression. The gene was digested with restriction enzymes, and 6.5 kb DNA of the upstream region containing a part of the structural gene encoding UCP-2 was obtained. From the DNA obtained, 3.5 kb

DNA containing the base sequence deduced to be the 1st and 2nd exons (2.5 kb DNA as the 5' upstream region) were re-cloned in plasmid DNA.

A plasmid DNA was constructed by connecting luciferase gene as a reporter gene to downstream of the 3.5 kb DNA. Measuring the luciferase activity in transformants of HepG2 cells, UCP-2 promoter was found in the 3.3 kb DNA of the upstream region of the UCP-2 structural gene. As a result of detailed analysis, the regulator sequence that may control the expression of UCP-2 was found.

The inventors proceeded the study based on these findings, and completed the present invention.

[0005]

The present invention relates to the followings:

- (1) A DNA containing uncoupling protein-2 (UCP-2) promoter region containing the regulator sequence;
- (2) A DNA described in (1) wherein the regulator sequence is a sequence containing peroxisome proliferator response element (PPRE);
- (3) A DNA described in (1) wherein the regulator sequence is a sequence containing CCAAT/enhancer binding protein (C/EBP) binding sequence;
- (4) A DNA described in (1) wherein the promoter region is a base sequence presented by position 1 to 2270 of SEQ ID NO: 1 or a base sequence containing a part of the said base sequence;
- (5) A DNA described in (1) wherein the DNA is a recombinant vector containing DNA having a structural gene under control of UCP-2 promoter region containing a regulator sequence;
- (6) A transformant transformed by a DNA described in (1);
- (7) A method for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of a transformant described in (6);

(8) A method for screening an antiobestic drug, an antidiabetic drug, a depressor, an antihyperlipemic drug, and an antipyretic drug characterized by use of a transformant described in (6);

(9) A kit for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of a transformant described in (6); and

(10) A pharmaceutical composition containing a compound or its salt that promotes or inhibits UCP-2 promoter activity obtained using a screening method described in (7) or a screening kit described in (9).

[0006]

[MODE OF EMBODIMENT OF THE INVENTION]

A DNA containing the UCP-2 promoter region containing the regulator sequence of this invention may be any DNA containing the regulator sequence described below with UCP-2 promoter activity.

Specifically, a DNA of this invention may be any DNA containing the base sequence presented by position 1 to 2270 of SEQ ID NO: 1 or a part of said sequence.

A DNA of this invention may be (i) genomic DNA, (ii) cDNA, and synthetic DNA derived from human cells (e.g. hepatocytes, splenocytes, neurocytes, glial cells, pancreatic β cells, bone marrow cells, mesangium cells, Langerhans' cells, epidermal cells, epithelial cells, endothelial cells, fibroblasts, fibre cells, muscle cells, fat cells, immune cells (e.g. macrophages, T cells, B cells, natural killer cells, mast cells, neutrophils, basophils, eosinophils, monocytes), megakaryocytes, synovial cells, chondrocytes, osteocytes, osteoblasts, osteoclasts, mammary cells, and interstitial cells, or precursor cells, stem cells, or cancer cells of said cells, and any tissue in which said cells are present, for example, the brain, each region of the brain (e.g. olfactory bulbs, amygdaloid nucleus, basal ganglia, hippocampus, thalamus, hypothalamus,

cerebral cortex, medulla oblongata, cerebellum), spinal cord, pituitary gland, stomach, pancreas, kidneys, liver, gonads, thyroid gland, gallbladder, bone marrow, adrenal glands, skin, muscle, lung, digestive tract (e.g. large intestine, small intestine), blood vessels, heart, thymus, spleen, salivary glands, peripheral blood, prostate, testes, ovaries, placenta, uterus, bones, cartilages, joints, and skeletal muscles.

Specifically, a recombinant DNA containing the human UCP-2 promoter region of this invention can be obtained as follows.

Using the base sequence corresponding to the previously reported amino acid sequences of human UCP cDNA (Fleury, C. et al. (1997), Nature Genet. Vol. 15, 269-272) as the probes, for example, human genomic library inserted in EMBL3 vector is screened by publicly known method, and λ phage clones that react with the probes are obtained. A DNA is extracted from these phage clones, and the restriction enzyme map of the human gene inserted in the clones is prepared. DNA fragments are prepared by digestion with restriction enzymes, and the fragments react with the probes for the most upstream region of the cDNA are re-cloned in vectors for animal cells such as pCD vector, cDM8 vector (Aruffo, A. and Seed, B. (1987), Proc. Natl. Acad. Sci. USA, 84, 8573-8577), and retrovirus vector (Cone, R.D. and Mulligan, R.C. (1984), Proc. Natl. Acad. Sci. USA, 81, 6349-6353), and *Escherichia coli* plasmids such as pUC vector (Vieira, J. and Messing, J. (1987), Methods in Enzymology, 153, 3-11), and pCR-blunt vector (Ausubel, F.M. et al. (1994), Current Protocols in Molecular Biology), but not limited to these vectors. The base sequences of the cloned DNA are determined, and the position of the translation initiation codon on the gene can be determined by, for example, comparing the base sequence with the cDNA sequence. The position of the transcriptional initiation site on the gene can also be determined by comparing the

base sequence with the 5' end of known cDNA. By investigating motifs in the entire sequence, the binding site of known transcriptional regulatory factors can be determined.

The obtained DNA can be used without modification or if necessary, after digestion with restriction enzymes or being bound by linkers.

To measure the promoter activity, a detectable structural gene may be connected in downstream of the promoter region. For the structural gene connected in downstream of the promoter region, various reporter genes are used. For the reporter gene, luciferase, chloramphenicol acetyltransferase (CAT) gene, alkaline phosphatase gene, and β -galactosidase gene are commonly used, but any other structural genes for which a method of detecting the gene product is available may be used. To insert the above structural gene into the vector, the structural gene is ligated to an appropriate restriction enzyme site located downstream of the promoter region in the correct transcriptional orientation.

[0007]

For the host transformed by the recombinant vector described above, for example, *Escherichia* genus, *Bacillus* genus, Yeast, insect cells, insects, and animal cells are used.

Specific examples of the host *Escherichia* genus are *Escherichia coli* K12·DH1 [Proceedings of the National Academy of Sciences of the USA (Proc. Natl. Acad. Sci. USA), Vol. 60, 160 (1968)], JM103 [Nucleic Acids Research, Vol. 9, 309 (1981)], JM109, JA221 [Journal of Molecular Biology, Vol. 120, 517 (1978)], HB101 [Journal of Molecular Biology, Vol. 41, 459 (1969)], and C600 [Genetics, Vol. 39, 440 (1954)].

For the host *Bacillus* genus, for example, *Bacillus subtilis* MI114 [Gene, Vol. 24, 255 (1983)] and 207-21 [Journal of Biochemistry, Vol. 95, 87 (1984)] are used.

For the host yeast, for example, *Saccharomyces cerevisiae* AH22, AH22R, NA87-11A, DKD-5D, 20B-12, *Schizosaccharomyces pombe* NCYC1913, NCYC2036, and *Pichia pastoris* are used.

For the host insect cells, for example, when the virus is AcNPV, *Spodoptera frugiperda* cells (Sf cells), MG1 cells derived from the middle gut of *Trichoplusia ni*, High Five™ cells derived from *Trichoplusia ni* eggs, Mamestra brassicae-derived cells, and *Estigmena acrea*-derived cells are used. When the virus is BmNPV, silkworm-derived cell line *Bombyx mori* N (BmN cells) are used. For said Sf cells, for example, Sf9 cells (ATCC CRL1711), Sf21 cells (Vaughn, J.L. et al., *In Vivo*, 13, 213-217 (1977)) are used.

For the host insect, for example, silkworm larvae are used [Maeda et al., *Nature*, Vol. 315, 592 (1985)].

For the host animal cells, for example, monkey COS-7 cells, Vero, Chinese hamster CHO cells (CHO), dhfr gene-deficient Chinese hamster cells CHO (CHO (dhfr⁻) cells), mouse L cells, mouse AtT-20, mouse myeloma cells, rat GH3, human FL cells, white fat cells, egg cells, ES cells (Evans, M.J. and Kaufman, K.H. (1981), *Nature*, 292, 154) are used.

Animal cells, especially white fat cells, may be used. As a process of DNA transfer to individual animals, egg cells and ES cells (Evans, M.J. and Kaufman, K.H. (1981), *Nature*, 292, 154) are used.

For the method of transforming these cells, the calcium phosphate method (Graham et al. (1973), *Virology*, 52, 456), electroporation (Ishizaki et al. (1986), *Cell Engineering* (Saibo Kogaku), 5, 577), and microinjection are used.

More specifically, for transformation of bacteria of *Escherichia* genus, for example, the methods published in *Proc. Natl. Acad. USA*, Vol. 69, 2110 (1972) and *Gene*, Vol. 17, 107 (1982) are used.

Bacteria of *Bacillus* genus can be transformed following, for example, the method published in *Molecular &*

General Genetics, Vol. 168, 111 (1979).

Yeast can be transformed following, for example, the methods published in Methods in Enzymology, Vol. 194, 182-187 (1991) and Proc. Natl. Acad. USA, Vol. 75, 1929 (1978).

Insect cells and insects can be transformed following, for example, the method published in Bio/Technology, 6, 47-55 (1988).

Animal cells can be transformed by, for example, the methods described in Cell Engineering (Saibo Kogaku), Separate Vol. 8, New Cell Engineering Experimental Protocol, 263-267 (1995) (Shujun-sha) and Virology, Vol. 52, 456 (1973).

The transformant described above is cultured in the presence of the specified compound, and by measuring and comparing the gene product in the cultured material, the ability of controlling the promoter activity of the compound can be examined.

[0008]

The transformant is cultured by publicly known methods. For the medium for culturing the transformant using *Escherichia* and *Bacillus* hosts, liquid medium is appropriate, which contains carbon source, nitrogen source, inorganic compounds, and other substances necessary for the growth of the transformants. The carbon source includes, for example, glucose, dextrin, soluble starch, and sucrose, etc. The nitrogen source includes, for example, inorganic and organic compounds such as ammonium salts, nitrates, cornsteep liquor, peptone, casein, meat extract, soybean cake, and potato extract, etc. The inorganic compounds include, for example, calcium chloride, sodium dihydrogen phosphate, and magnesium chloride, etc. Yeast extract, vitamins, and growth-stimulating factors may be added. The pH about 5 - 8 is desirable for the culture medium.

For the culture medium for bacteria of *Escherichia* genus, for example, M9 medium containing glucose and casamino acid (Miller, Journal of Experiments in Molecular

Genetics, 431-433, Cold Spring Harbor Laboratory, New York, 1972) is preferred. When a higher efficiency of the promoter is required, reagent such as 3- β -indolylacrylic acid may be added. When the host is bacteria of *Escherichia* genus, the bacteria are generally cultured at about 15 - 43°C for about 3 - 24 hours, and aeration or stirring may be added to the culture if necessary.

When the host is bacteria of *Bacillus* genus, the bacteria are generally cultured at about 30 - 40°C for about 6 - 24 hours, and aeration or stirring may be added to the culture if necessary.

For the medium for culturing the transformant in yeast host, for example, Burkholder minimum medium [Bostian, K.L. et al., Proc. Natl. Acad. Sci. USA, Vol. 77, 4505 (1980)] and SD medium containing 0.5% casamino acid [Bitter, G.A. et al., Proc. Natl. Acad. Sci. USA, Vol. 81, 5330 (1984)] are used. The pH of the medium is preferably adjusted to about 5 - 8. The culture conditions are generally about 20 - 35°C for about 24 - 72 hours, and aeration or stirring may be added to the culture if necessary.

For the medium for culture of the transformants in insect cells and insect hosts, Grace's insect medium [Grace, T.C.C., Nature, 195, 788 (1962)] containing appropriate supplements such as inactivated 10% bovine serum is used. The pH of the medium is preferably adjusted to about 6.2 - 6.4. Usually, the culture conditions are at about 27°C for about 3 - 5 days, and aeration or stirring may be added to the culture if necessary.

For the culture medium of the transformants in animal cell hosts, for example, MEM containing about 5 - 20% fetal calf serum [Science, Vol. 122, 501 (1952)], DMEM [Virology, Vol. 8, 396 (1959)], RPMI 1640 medium [The Journal of the American Medical Association, Vol. 199, 519 (1967)], and 199 medium [Proceeding of the Society for the Biological Medicine, Vol. 73, 1 (1950)] are used. The pH is preferably adjusted to about 6 - 8. Usually, the culture

conditions are about 30 - 40°C for about 15 - 60 hours, and aeration or stirring may be added to the culture if necessary.

[0009]

Specifically, the regulator sequence may be any sequence of the base sequence presented by position from 1 to 2270 of SEQ ID NO: 1 to which the UCP-2 transcriptional regulatory factor can bind, such as sequences containing peroxisome proliferator response element (PPRE) presented by position 284 to 296 of SEQ ID NO: 1, sequences containing CCAAT/enhancer binding protein (C/EBP) binding sequence presented by position 1316 to 1320, 1364 to 1368, or 1698 to 1692 of SEQ ID NO: 1, sequences containing glucocorticoid response element (GRE) presented by position 753 to 758, 1023 to 1030, or 1450 to 1455 of SEQ ID NO: 1, and sequences containing MyoD presented by position 1428 to 1437 of SEQ ID NO: 1.

Therefore, a DNA of this invention contains the promoter region containing the said regulator sequence, and a DNA of this invention may contain a multiple number of the said regulator sequences.

For the base sequences containing a part of the base sequence presented by position 1 to 2270 of SEQ ID NO: 1, any base sequences containing the regulator sequence described above may be used. Specifically, the base sequence presented by position 255 to 430 of SEQ ID NO: 1, the base sequence presented by position 255 to 717 of SEQ ID NO: 1, the base sequence presented by position 717 to 1133 of SEQ ID NO: 1, the base sequence presented by position 1133 to 1389 of SEQ ID NO: 1, and the base sequence presented by position 255 to 1857 of SEQ ID NO: 1 are used.

[0010]

Since a DNA of this invention contains the UCP-2 promoter region containing the regulator sequence, using the transformant described above, a compound or its salt

that promotes or inhibits UCP-2 promoter activity can be screened. The said screening method, screening kit, and the said compound that promotes or inhibits UCP-2 promoter activity obtained using the said screening method and screening kit are specifically explained below.

(1) A method for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity

A transformant transformed by the DNA of this invention described above is useful for searching and determining a compound or its salt that promotes or inhibits UCP-2 promoter activity of this invention.

A method for determining a compound or its salt that promotes or inhibits UCP-2 promoter activity of this invention is characterized by measuring and comparing polypeptide expression between a transformant of this invention contacted to test compound and the transformant lacking the UCP-2 promoter region of this invention contacted to the test compound.

The said test compound includes peptides, proteins, non-peptide compounds, synthetic compounds, and fermentation products, etc., and these test compounds may be novel compounds or known compounds.

For the polypeptide to be expressed, polypeptides encoded by the structural genes described above (preferably reporter genes) are used.

For the measurement method of polypeptide expression, for example, measurement of luciferase activity according to the method described by Brasier, A.R. et al. (1989) in Biotechniques Vol. 7, 1116-1122, is used.

[0011]

(2) A kit for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity

A kit for determining a compound or its salt that promotes or inhibits UCP-2 promoter activity (e.g. a compound that promotes or inhibits heat production) is characterized by use of the transformant described above.

Examples of the kit for determining a compound or its salt that promotes or inhibits UCP-2 promoter activity of this invention are as follows.

① Screening reagents

1. Cell culture medium

Dulbecco's modified Eagle's medium (Gibco Co.) supplemented with 10% fetal calf serum (Gibco Co.)

2. Cell differentiation medium

Dulbecco's modified Eagle's medium (Gibco Co.) supplemented with 5% rabbit serum (Gibco Co.)

3. Plasmid for measurement of UCP-2 promoter activity

pGL3-basic (Promega Co.) plasmid DNA carrying UCP-2 promoter sequence of this invention and a structural gene (e.g. luciferase gene) inserted downstream of the UCP-2 promoter

4. Host cell line

MG-63 cells (osteosarcoma cell line, obtained from ATCC)

5. Test compounds

Aqueous solutions are stored at 4°C or -20°C, and diluted to 1 μ M with cell differentiation medium at use. Test compounds that are slightly soluble in water are dissolved in dimethylformamide, DMSO, and methanol.

② Screening method

Host cells are seeded in 96-well microplates at a density of 1×10^5 cells/well, and cultured in an incubator at 37°C in 5% CO₂ overnight.

The cells are transfected with 1 μ g/well of plasmid for UCP-2 promoter activity measurement.

One hour after transfection, 0.1 ml of test compound is added to each well, and the cells are cultured in an incubator at 37°C in 5% CO₂ for 48 hours.

After culture, 0.1 ml of PicaGene LT (Toyo Ink Co.) is added to each well, stirred for five minutes, and then the luminescence is measured using a 96-plate measurement system (Amersham-Pharmacia Co.).

(3) A compound or its salt that promotes or inhibits UCP-2 promoter activity obtained using the screening method described in (1) and the screening kit described in (2)

If a compound that promotes or inhibits UCP-2 promoter activity is found using the screening method described in (1) or the screening kit described in (2), the compound may be used as a prophylactic or therapeutic drug for obesity syndrome, and thus, the compound may be used as a radical therapeutic drug for lifestyle diseases (diabetes, hypertension, hyperlipidemia). Therefore, the compound may be used as an antiobestic drug, an antidiabetic drug, a depressor, and an antihyperlipemic drug.

When the compound reduces or inhibits the promoter activity, the compound may be used as an antipyretic drug because the compound decreases or inhibits heat production.

[0012]

When the said compound is used as prophylactic and/or therapeutic drugs for the diseases described above, the preparation can be obtained by the conventional methods.

For example, the said compound can be orally administered as sugar coated tablet, capsule, elixir, and microcapsule, etc., or non-orally as injection such as aseptic solution in water or other pharmaceutically acceptable liquid and suspension. Preparations can be manufactured by, for example, mixing with physiologically acceptable known carrier, flavor, filler, vehicle, antiseptic, stabilizer, and binder in a unit-dosage form required for generally approved drug preparation. The amount of the active ingredient is set to prepare an appropriate dosage within the specified range.

For the additive miscible with tablets and capsules, for example, binders such as gelatin, cornstarch, tragacanth and acacia, fillers such as crystalline cellulose, swellings such as cornstarch, gelatin, and alginic acid, lubricants such as magnesium stearate, sweeteners such as sucrose, lactose and saccharin, and

flavors such as peppermint, akamono oil and cherry are used. When the unit-dosage form is capsule, liquid carrier such as fat and oil may be contained. Aseptic compositions for injection can be formulated following the usual preparation procedure such as dissolving or suspending the active substance in vehicle, e.g. water for injection, and natural plant oils e.g. sesame oil and coconut oil. For the aqueous solution for injection, for example, physiological saline and isotonic solutions (e.g. D-sorbitol, D-mannitol, sodium hydrochloride) containing glucose and other adjuvant is used. Appropriate dissolution-assisting agents, for example, alcohol (e.g. ethanol), polyalcohol (e.g. propylene glycol, polyethylene glycol), and nonionic surfactant (e.g. polysorbate 80(TM), HCO-50) may be combined. For the oily solution, for example, sesame oil and soybean oil are used, and dissolution-assisting agents such as benzyl benzoate and benzyl alcohol may be combined.

[0013]

The prophylactic/therapeutic drugs described above may be combined with, for example, buffers (e.g. phosphate buffer, sodium acetate buffer), soothing agents (e.g. benzalkonium chloride, procaine hydrochloride), stabilizers (e.g. human serum albumin, polyethylene glycol), preservatives (e.g. benzylalcohol, phenol), and antioxidants. The preparation for injection is usually filled in appropriate ampoules.

The preparations obtained as described above are safe and low toxic, and can be administered to, for example, human and mammals (e.g. rats, mice, rabbits, sheep, pigs, cattle, cats, dogs, monkeys, etc.).

The dosage of the said compound or its salt differs depending on the target individual, target organ, symptoms, and administration method, etc. When it is administered orally, in general, for adults (60 kg body weight), about 0.1 - 100 mg per day, preferably about 1.0 - 50 mg per day, more preferably about 1.0 - 20 mg per day is administered.

When it is administered non-orally, the dosage per dosing differs depending on the target individual, target organ, symptoms, and administration method, etc. For example, in case of injection in general, for adults (60 kg body weight), it is desirable to intravenously inject about 0.01 - 30 mg per day, preferably about 0.1 - 20 mg per day, more preferably about 0.1 - 10 mg per day. Converting the dosage for 60 kg, the said compound or its salt can be administered to other animals.

[0014]

In this specification and drawings, the codes of bases and amino acids are according to IUPAC-IUB Commission on Biochemical Nomenclature or common codes in the art. The examples are shown below. For amino acids that may have the optical isomer, L form is presented unless it is specified.

[0015]

DNA : deoxyribonucleic acid
cDNA : complementary deoxyribonucleic acid
A : adenine
T : thymine
G : guanine
C : cytosine

[0016]

The SEQ ID NOs shown in the Sequence Listing of this Specification present the sequences below.

[SEQ ID NO: 1] Base sequence of cDNA containing human UCP-2 promoter region cloned in Example 1.

[SEQ ID NO: 2] Synthetic DNA used in screening of cDNA containing human UCP-2 promoter region.

[SEQ ID NO: 3] Synthetic DNA used in screening of cDNA containing human UCP-2 promoter region.

[SEQ ID NO: 4] Synthetic DNA used in screening of cDNA containing human UCP-2 promoter region.

[SEQ ID NO: 5] Synthetic DNA used in screening of cDNA containing human UCP-2 promoter region.

[0017]

[EXAMPLES]

The present invention is explained in detail below showing examples, but it is not intended to limit the scope of this invention to the description.

Escherichia coli transformant TOP10/pCR-ucp2p5'#1-10 obtained in the Example 1 described below was deposited with the Ministry of International Trade and Industry, Agency of Industrial Science and Technology, National Institute of Bioscience and Human Technology (NIBH) as deposit number FERM BP-6587 on November 24, 1998 and with Institute for Fermentation, Osaka (IFO) as deposit number IFO 16219 on November 11, 1998.

The present invention is explained in detail below showing examples, but it is not intended to limit the scope of this invention to the description.

[0018]

Example 1 Cloning of human UCP-2 cDNA

Using 0.5 ng of human kidney cDNA (Clontech Laboratory, California, USA) as the template and the base sequence of base number 55 to 82: 5'-ATGGTTGGGTTCAAGGCCACAGATGTGCCC-3' of previously reported human UCP-2 cDNA [Gimeno, R. et al. (1997), Diabetes, Vol. 46, 900-906] and the base sequence complementary to base number 1300 to 1329: 5'-ATACAGGCCGATGCGGACAGAGGCAAAGCT-3' as oligonucleotide primers, human UCP-2 gene was amplified by PCR (after heating at 94°C for 5 min., a cycle consisting of heating at 94°C for 1 min, 55°C for 0.5 min and 72°C for 1.5 min was repeated 30 times, followed by heating at 72°C for 5 min), then inserted into pCR-blunt vector. Using this plasmid DNA carrying the insert as the template, oligonucleotide primers were prepared, and probes were prepared using PCR DIG probe synthesis kit (Boehringer-Mannheim Co.) following the attached instruction. Using the prepared probes, human

genomic DNA library (Clontech Laboratory, California, USA) in 3×10^6 phages was screened using nitrocellulose filters. Plaque hybridization was performed using DIG Easy hyb (Boehringer-Mannheim Co.), DIG Wash and Block Buffer Set (Boehringer-Mannheim Co.), and DIG nucleic acid detection kit (Boehringer-Mannheim Co.) following the attached instruction. As a result, eight positive clones were obtained from 3×10^6 phages. Of these clones, an inner primer of non-coding exon of previously reported human UCP-2 cDNA sequence [Gimeno, R.E. et al. (1998), Diabetes, 47 (4), 685-687] was synthesized (5'-CAAAGCTGCCAGTGGCTATCATGGCCCG-3'), and a clone containing the non-coding exon was detected by PCR using a primer containing EMBL3 sequence (5'-GACCGGTCGACCCAGATCTGGGTCGACCTG-3'), and a genomic clone containing the 5' upstream region of UCP-2 was obtained. From the genomic clone, 3.5 kbp fragment containing UCP-2 promoter region was prepared, and inserted into pCR-blunt vector (Invitrogen Co.), and transformant *E. coli* TOP/10 pCR-UCP2P5' #1-10 was prepared. After that, the restriction enzyme map was prepared and the base sequence was determined. The determined base sequence is shown in Figures 1 to 6. As shown in Figures 1 to 6, base number 2271 - 2326 and 3416 - 3505 were completely consistent with human UCP-2 cDNA (Gimeno, R.E. et al. (1998), Diabetes, 47 (4), 685-687). Furthermore, the terminal base sequences of the consistent regions were consistent with Shahnborn rule, which is the characteristic of intron-exon boundary base sequence, suggesting that the consistent base sequences are introns. A sequence likely to be CpG island (base number about 2070 - 2270), which is a characteristic of promoters without containing TATA-box sequence, was also confirmed upstream of the first exon. In the promoter sequence described above, PPRE (base number 284 - 296), which is the regulator sequence of promoters of fat cell-related genes, and three C/EBP binding sites (base number 1316 - 1320,

1364 - 1368, 1698 - 1692) were confirmed.

[0019]

Example 2 Examination of human UCP-2 gene promoter activity

To confirm the promoter activity of the cloned genomic DNA fragment, luciferase assay was performed. pGL3-Basic plasmid (Promega Co.) carrying firefly luciferase gene as the reporter gene was used for the vector. As the internal standard, pRL-SV40 plasmid (Promega Co.) expressing sea pansy luciferase under control of SV40 promoter was used.

EcoRI fragment (3.5 kb) was isolated from the genomic human UCP-2 DNA and blunted using Blunting High Kit (TOYOBO Co.), and then ligated to SmaI-digested pGL3-Basic plasmid DNA. Following the above procedure, human UCP-2 promoter/luciferase vector was constructed in which the base number 1 - 3505 shown in Figures 1 to 6 was inserted into pGL3-Basic vector. The constructed human UCP-2 promoter/luciferase vector was transiently transfected in HepG2, the human liver cancer cell line, in which constant expression of UCP-2 was confirmed by RT-PCR, and the activity was examined.

HepG2 cells were seeded in 24-well multiplates (Nunk Co.) at a density of 60,000 cells/well, and cultured at 37°C in 5% CO₂ overnight. Using SuperFect Transfection Reagent (QIAGEN Co.), cells were transiently transfected with 1 µg of human UCP-2 promoter/luciferase vector DNA or pGL3-Basic DNA and 0.1 µg of pRL-SV40 DNA. The procedure was performed according to the attached instruction. Then, the cells were cultured at 37°C in 5% CO₂ for 24 hours, and the luciferase activity was detected using PicaGene Dual Sea Pansy (Nippon Gene Co.) according to the attached instruction. The measurement data were presented as relative activity to the internal standard value of pRL-SV40-derived sea pansy luciferase activity. The results are shown in Figure 7. The human UCP-2 promoter/luciferase

vector-derived luciferase activity was markedly higher than that of pGL3-Basic lacking the promoter. Therefore, the genomic DNA of human UCP-2 gene of this invention has a promoter activity reflecting the in vivo UCP-2 gene expression system.

[0025]

[Meritorious effects of the invention]

Since UCP-2 promoter of this invention contains the regulator sequence, it has higher activity reflecting the in vivo UCP-2 DNA expression system in human than the promoter lacking the regulator sequence. Therefore, the UCP-2 promoter of this invention can be used as a promoter inserted in vectors for treatment of human diseases and setting drug-screening systems under conditions closer to in vivo environment in human.

[0020]

[SEQUENCE LISTING]

[SEQ ID NO: 1]

Length: 3505

Type: DNA

Number of strands: double-stranded

Topology: linear

Sequence: cDNA

Sequence:

```
AACGGATCTG CCCGCCTCAG CCTCCCAAAG TGCTGGGATT GCAGGCGTGA GCCACCTCAC   60
CTGGCTACAA GTTTTCAAAA TACATTTATC TAGTACCCAT ACATTCTCCA GTTTGTCCAC  120
AGGACATCTT ATGACTTGAG CAAGCTGCTA AAAATCCAAG GGTGCAGCGT TTGTATGTCT  180
ATAGGATTGC TCAGATCTGC CCCCACCCTG AAAGAATTTA AGAGAATTTC TTGAGGCCAG  240
GCACAGTGGC TCACACCTGT AATTCCAGTA CTGTGAGAGT CCGAGGTCAG AGGACTGCTT  300
GAGGCCAGGA GTTCAAGAGC AGCCTGGACA ACACAGGGAG ACCTGTCACT ACAAAGAATA  360
AATAAATTAG CCAGGCTTAG TGGCTCATCC CTGTGGTCCC AGCTACTAGG GAGGCAGAAG  420
TAGGACTGCT TGTCCCAGGA GGTCAAGACT GCAGTGAGCT GAGACCCAGC CACCTGCATT  480
CCAGCCTGGG CAACAAAAAG AGACCCTGTC TCAAAAAATA AGTTAAATAA ATAAATAATA  540
AAAATAGTTT AAACCCTAAA CACATCTTCT TTTTCAAAGA GGACTTCTTA AGGACTTCAT  600
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GCTGCGTCCT GTTGATCTCC ACTTCCCTTT TTCAGCGTCC ACACTTTTAA CAGTCTCTTT 660
TGCCAAGGAT AATAAGTATA TAGTTTCTGG AATCCAGATT CTTCCCTGTT TGGACAGCCA 720
GGGGGACAAT TTTTGGTCTG CAGGCCTTTG CATCTGTTCT GCTGTTGCTC AGCAATCTCA 780
CAGCAAATTT GCCGAGCCTC TCCGGAATGC ACAGCCAGAC AGAGCTCAGC GCAAAAAGCTA 840
GAGAACCTGG CGGAGGGAGA CTCACAGTGC CACAAAAAAA CTTTATCTTT TCTTTTTTTT 900
TTTCTTTTCT TTCTTTCTCT TTCTTTCTTG TCTTTCTGTC TTTCCTCTCT CTCTCTCTGT 960
CTTTCTTTCC TCTCTTTCTT TCTTTTTTCC TACATGGCAA GATCTCCTCA TGGCAGAAAT 1020
AATCTGCCTT GACTTCTGTT TCCACGCTGC TTCTGCCAGG ACCATGCGCT CGGCGTGTTT 1080
TTCTTTCCGC TATAATTATC CAGGCCCATC CCAGCTCTGG TCCCCTCAGC TGTTCCCTGG 1140
CAGTCCCTTC TGCTGGTGAA AACACATATG GCGCCGGCCT GACCAGGGTG TAAGTGTGTG 1200
AATATCAGGA AGATGACTGA ACGTCTTTGG GACTCCGTTT CCTCATTGTA AAATGGAGGT 1260
TAATACCAGC CTTCTTCTAC TCCCCAAACG CACGTGTTTG TCCCGGCCAG AGGGCCCAAT 1320
TGTTGGCTGT TCACGCATCA GTTACCCCCA CAGGACGGGT CAGCCAATTA AAGGCGAACC 1380
AGGCCCCGTC CATCTCCTGA CGCCTTTTCT CATCCCAGGG CTGGACAGGC AGCTGGCCTG 1440
GGCCCCGCTC TGCCTTGTCA CGTGCGGGGG CCGGCCCGTT TGCTTGTCTG TGTGTAGGAG 1500
CGTGAGGTCA CGCTGGGTGC TCCCGCCCCG CCGGGGCGTT TAGTGTCCCT GGTCCCTAAA 1560
CGCCAGGCCG CTCCACCGGG GGAGAAGGCG CGAACCCAG CCGAGCCCAA CGGCTGTTGT 1620
CGGTTGCCGG GCCACCTGTT GCTGCAGTTC TGATTGGTTC CTTCCCCCGA CAACGCGGCG 1680
GCTGTAACCA ATCGACAGCG AGGCCGGTCG CGAGGCCCCA GTCCCGCCCT GCAGGAGCCA 1740
GCCGCGCGCT CGCTCGCAGG AGGGTGGGTA GTTTGCCCAG CGTAGGGGGG CTGGGCCCCAT 1800
AAAAGAGGAA GTGCACTTAA GACACGGCCC CGCTGGACGC TTGTTAGAAA CCGTCCTGGC 1860
TGGGAAGGCA AGAGGTGTGT GACTGGACAA GACTTGT TTC TGGCGGTCAG TCTTGCCATC 1920
CTCACAGAGG TTGGCGGCCC GAGAGAGTGT GAGGCAGAGG CGGGGAGTGG CAAGGGAGTG 1980
ACCATCTCGG GGAACGAAGG AGTAAACGCG GTGATGGGAC GCACGGAAC GGGAGTGGAG 2040
AAAGTCATGG AGAGAACCTT AGGCGGGGCG GTCCCCGCGG AAAGGCGGCT GCTCCAGGGT 2100
CTCCGCACCC AAGTAGGAGC TGGCAGGCCG GGCCCCGCCC CGCAGGCCCC ACCCCGGGCC 2160
CCGCCCCCGA GGCTTAAGCC GCGCCGCCGC CTGCGCGGAG CCCCCTGCG AAGCCCAGCT 2220
GCGCGCGCCT TGGGATTGAC TGTCCACGCT CGCCCGGCTC GTCCGACGCG CCCTCCGCCA 2280
GCCGACAGAC ACAGCCGCAC GCACTGCCGT GTTCTCCCTG CGGCTCGGTG AGCCTGGCCC 2340
CAGCCCTGCG CCCTTTGCGC CCCCCACGCT TGTCTGCGT GCGCTGCCCC CTCTTCCATT 2400
TACCTTCTCT CCCACCCAAG TTTGTACTCT TTTCTTTCTC TCGGTTTTAT TTTTGTGTTT 2460
TGTTTGTGTTG TTTGAGACAG GCTTTCGCTC TGTCTCCCAG GCTGGAGTGC AGTGGCGCGA 2520
TCTCGGCTCA CTGCAGCCTC CACCTCCCAG GTTCAAGCGA TCCGCCTGCC GAGTAGCTGG 2580
GATTACAGGC GCCCGCCACC ACGCCTGGCT AATTTTTGTG TTTTGTAGAG ATGGGGTTTC 2640
GCCATGTTGG CCAGGCTGGC CTCGAACTGC TCAGCTCAAG CAATCCGCCC GCCTCGGCCT 2700
CACAAAGTCC TAGAATTTTA GGCATGAGCC TCCGGGTCCG GCCTGTGCTA ATCCTTTCTG 2760

TCCTTGGTTC TTTATTTCCC TTCTCTCTTT TTCTTAGTCC CTTTTGTTCT TTCCCTCTCC 2820
 CGTTCAGTTG GCTGTCGTTT GAGCCTCCAC CTTTTCAC TCCTCCTTTCC ACCACGATGC 2880
 CGAGCCCTGC CTGGGATGGG GACCATCAGC GATGACCACA ATGACCTCTC CTTACCAGG 2920
 CAGCTCCAGG CAGTGTTCCT GCACCGCCTT TCCCAAGGCT TGGGGGCTTT TTCTAGTGGG 3000
 CTTTGAGCTG CTCAATCTGG CCTCTGCAGG GCCGGCTCCC AGCCCTTCCA ACCTCCTCAC 3060
 AGCCCCGACCT GGGACCTAGC CAATTCCCGG AGAGTCTCTG TCCCATCGTG ACCCCCTCAC 3120
 AACTCTCCCA CTCACCAAAG TCTGATGACT GTGCTAGGGG GTGCTTATAT AGAGTACTGA 3180
 GTGTTACAAA AGCAGAAGTC TGGATGAGAA CCAATTTGTG ATATTAAGCA GGTGGGGTGG 3240
 GGGTGGGGAG TGTACCTAGG TTCATTTTCC GCCCTGCTTT TCCCCTTTCC AGTGTGTGCA 3300
 CTTAACCAGT CCCTGGGCCC TGTTCCTCAT CCCCTCCAA GGCATGGATT GGGTGGGCTT 3360
 GTGTGTCTTG GGGCAGGTGG CCCTTTCTAA ACTCTCTGCC TTTGCTCACC CACAGGACAC 3420
 ATAGTATGAC CATTAGGTGT TTCGTCTCCC ACCCATTTTC TATGGAAAAC CAAGGGGATC 3480
 GGGCCATGAT AGCCACTGGC AGCTT 3505

[0021]

[SEQ ID NO: 2]

Length: 30

Type: DNA

Number of strands: single-stranded

Topology: linear

Sequence: other nucleic acid, synthetic DNA

Sequence:

ATGGTTGGGT TCAAGGCCAC AGATGTGCCC 30

[0022]

[SEQ ID NO: 3]

Length: 30

Type: DNA

Number of strands: single-stranded

Topology: linear

Sequence: other nucleic acid, sybthetic DNA

Sequence:

ATACAGGCCG ATGCGGACAG AGGCAAAGCT 30

[0023]

[SEQUENCE LISTING]

[SEQ ID NO: 4]

Length: 28

Type: DNA

Number of strands: single-stranded

Topology: linear

Sequence: other nucleic acid, synthetic DNA

Sequence:

CAAAGCTGCC AGTGGCTATC ATGGCCCG

28

[0024]

[SEQUENCE LISTING]

[SEQ ID NO: 5]

Length: 30

Type: DNA

Number of strands: single-stranded

Topology: linear

Sequence: other nucleic acid, synthetic DNA

Sequence:

GACCGGTCGA CCCAGATCTG GGTCGACCTG

30

[0026]

[BRIEF DESCRIPTION OF THE DRAWINGS]

Figure 1 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1 (continued to Figure 2).

Figure 2 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1 (continued from Figure 1 to Figure 2).

Figure 3 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1 (continued from Figure 2 to Figure 4).

Figure 4 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1 (continued from Figure 3 to Figure 5).

Figure 5 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1

(continued from Figure 4 to Figure 6).

Figure 6 shows the base sequence of cDNA containing the human UCP-2 promoter region cloned in Example 1 (continued from Figure 5).

Figure 7 shows the luciferase activity measured in Example 2.

[Document] ABSTRACT

[Abstract]

[Problem to be solved]

This invention provides a DNA containing UCP-2 promoter region containing the regulator sequence.

[Means for solving problem]

This invention relates to a DNA containing uncoupling protein-2 (UCP-2) promoter region containing the regulator sequence, a transformant transformed with the said DNA, a method for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of the said transformant, a method for screening an antiobestic drug, an antidiabetic drug, a depressor, an antihyperlipemic drug, and an antipyretic drug characterized by use of the said transformant, a kit for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity characterized by use of the said transformant, and pharmaceutical composition containing a compound or its salt that promotes or inhibits UCP-2 promoter activity obtained using the said screening method or the said screening kit.

[Meritorious effects]

Since UCP-2 promoter of this invention contains the regulator sequence, it has higher activity reflecting the in vivo UCP-2 expression system in human than the promoter lacking the regulator sequence. Therefore, the UCP-2 promoter of this invention can be used as a promoter inserted in vectors for treatment of human diseases and drug screening systems under conditions closer to in vivo environment in human.

[Document] Drawing

[Fig.1]

10 20 30 40 50 60
AACGGATCTG CCCGCCTCAG CCTCCCAAAG TGCTGGGATT GCAGGCGTGA GCCACCTCAC

70 80 90 100 110 120
CTGGCTACAA GTTTTCAAAA TACATTTATC TAGTACCCAT ACATTCTCCA GTTTGTCCAC

130 140 150 160 170 180
AGGACATCTT ATGACTTGAG CAAGCTGCTA AAAATCCAAG GGTGCAGCGT TTGTATGTCT

190 200 210 220 230 240
ATAGGATTGC TCAGATCTGC CCCCACCCTG AAAGAATTTA AGAGAATTTC TTGAGGCCAG

250 260 270 280 290 300
GCACAGTGGC TCACACCTGT AATTCCAGTA CTGTGAGAGT CCGAGGTCAG AGGACTGCTT

PPRE

310 320 330 340 350 360
GAGGCCAGGA GTTCAAGAGC AGCCTGGACA ACACAGGGAG ACCTGTCACT ACAAAGAATA

370 380 390 400 410 420
AATAAATTAG CCAGGCTTAG TGGCTCATCC CTGTGGTCCC AGCTACTAGG GAGGCAGAAG

430 440 450 460 470 480
TAGGACTGCT TGTCCCAGGA GGTCAAGACT GCAGTGAGCT GAGACCCAGC CACCTGCATT

490 500 510 520 530 540
CCAGCCTGGG CAACAAAAAG AGACCCTGTC TCAAAAAATA AGTTAAATAA ATAAATAATA

550 560 570 580 590 600
AAAATAGTTT AAACCCTAAA CACATCTTCT TTTTCAAAGA GGACTTCTTA AGGACTTCAT

610 620 630 640 650 660
GCTGCGTCCT GTTGATCTCC ACTTCCCTTT TTCAGCGTCC ACACTTTTAA CAGTCTCTTT

[Fig.2]

670	680	690	700	710	720
TGCCAAGGAT	AATAAGTATA	TAGTTTCTGG	AATCCAGATT	CTTCCCTGTT	TGGACAGCCA
730	740	750	760	770	780
GGGGGACAAT	TTTGGTCTG	CAGGCCTTTG	<u>CA</u> <u>TC</u> <u>TG</u> <u>TT</u> <u>CT</u>	GCTGTTGCTC	AGCAATCTCA
			GRE		
790	800	810	820	830	840
CAGCAAATTT	GCCGAGCCTC	TCCGGAATGC	ACAGCCAGAC	AGAGCTCAGC	GCAAAAGCTA
850	860	870	880	890	900
GAGAACCTGG	CGGAGGGAGA	CTCACAGTGC	CACAAAAAAA	CTTTATCTTT	TCTTTTTTTT
910	920	930	940	950	960
TTTCTTTTCT	TTCTTTCTCT	TTCTTTCTTG	TCTTTCGTGC	TTTCCTCTCT	CTCTCTCTGT
970	980	990	1000	1010	1020
CTTCTTTTCC	TCTCTTTCTT	TCTTTTTTCC	TACATGGCAA	GATCTCCTCA	TGGCAGAAAT
1030	1040	1050	1060	1070	1080
<u>AATCTGCC</u> <u>CTT</u>	GACTTCTGTT	TCCACGCTGC	TTCTGCCAGG	ACCATGCGCT	CGGCGTGTTT
GRE					
1090	1100	1110	1120	1130	1140
TTCTTTCCGC	TATAATTATC	CAGGCCCATC	CCAGCTCTGG	TCCCCTCAGC	TGTTCCCTGG
1150	1160	1170	1180	1190	1200
CAGTCCCTTC	TGCTGGTGAA	AACACATATG	GCGCCGGCCT	GACCAGGGTG	TAAGTGTGTG
1210	1220	1230	1240	1250	1260
AATATCAGGA	AGATGACTGA	ACGTCTTTGG	GACTCCGTTT	CCTCATTGTA	AAATGGAGGT

[Fig.3]

1270 1280 1290 1300 1310 1320
TAATACCAGC CTTCTTCTAC TCCCCAAACG CACGTGTTTG TCCCGGCCAG AGGGCCCAAT
C/EBP

1330 1340 1350 1360 1370 1380
TGTTGGCTGT TCACGCATCA GTTACCCCCA CAGGACGGGT CAGCCAATTA AAGGCGAACC
C/EBP

1390 1400 1410 1420 1430 1440
AGGCCCCGGTC CATCTCCTGA CGCCTTTTCT CATCCCAGGG CTGGACAGGC AGCTGGCCTG
MyoD

1450 1460 1470 1480 1490 1500
GGCCCCGGCTC TGCCTTGTCA CGTGCGGGGG CCGGCCCGTT TGCTTGTCTG TGTGTAGGAG
GRE

1510 1520 1530 1540 1550 1560
CGTGAGGTCA CGCTGGGTGC TCCCGCCCCG CCGGGGCCTT TAGTGTCCTT GGTCCCTAAA

1570 1580 1590 1600 1610 1620
CGCCAGGCCG CTCCACCGGG GGAGAAGGCG CGAACCCAG CCGAGCCCAA CGGCTGTTGT

1630 1640 1650 1660 1670 1680
CGGTTGCCGG GCCACCTGTT GCTGCAGTTC TGATTGGTTC CTTCCCCGA CAACGCGGCG

1690 1700 1710 1720 1730 1740
GCTGTAACCA ATCGACAGCG AGGCCGGTCG CGAGGCCCCA GTCCCGCCCT GCAGGAGCCA
C/EBP

1750 1760 1770 1780 1790 1800
GCCGCGCGCT CGCTCGCAGG AGGGTGGGTA GTTTGCCAG CGTAGGGGGG CTGGGCCCAT

1810 1820 1830 1840 1850 1860
AAAAGAGGAA GTGCACTTAA GACACGGCCC CGCTGGACGC TTGTTAGAAA CCGTCCTGGC

1870 1880 1890 1900 1910 1920
TGGGAAGGCA AGAGGTGTGT GACTGGACAA GACTTGTTTC TGGCGGTCAG TCTTGCCATC

【Fig.4】

1930	1940	1950	1960	1970	1980
CTCACAGAGG	TTGGCGGCC	GAGAGAGTGT	GAGGCAGAGG	CGGGGAGTGG	CAAGGGAGTG
1990	2000	2010	2020	2030	2040
ACCATCTCGG	GGAACGAAGG	AGTAAACGCG	GTGATGGGAC	GCACGGAAAC	GGGAGTGGAG
2050	2060	2070	2080	2090	2100
AAAGTCATGG	AGAGAACCCT	AGGCGGGGCG	GTCCCCGCGG	AAAGGCGGCT	GCTCCAGGGT
2110	2120	2130	2140	2150	2160
CTCCGCACCC	AAGTAGGAGC	TGGCAGGCCC	GGCCCCGCCC	CGCAGGCCCC	ACCCCGGGCC
2170	2180	2190	2200	2210	2220
CCGCCCCCGA	GGCTTAAGCC	GCGCCGCGCG	CTGCGCGGAG	CCCCACTGCG	AAGCCCAGCT
2230	2240	2250	2260	2270	2280
GCGCGCGCCT	TGGGATTGAC	TGTCCACGCT	CGCCCGGCTC	GTCCGACGCG	CCCTCCGCCA
2290	2300	2310	2320	2330	2340
GCCGACAGAC	ACAGCCGCAC	GCACTGCCGT	GTTCTCCCTG	CGGCTCGGTG	AGCCTGGCCC
2350	2360	2370	2380	2390	2400
CAGCCCTGCG	CCCTTTGCGC	CCCCCAGGCT	TGTTCTGCGT	GCGCTGCCCC	CTCTTCCATT
2410	2420	2430	2440	2450	2460
TACCTTCTCT	CCCACCCAAG	TTTGTACTCT	TTTCTTTCTC	TCGGTTTAT	TTTTTGTTTT
2470	2480	2490	2500	2510	2520
TGTTTGTTTG	TTTGAGACAG	GCTTTGCTC	TGTCTCCAG	GCTGGAGTGC	AGTGGCGCGA
2530	2540	2550	2560	2570	2580
TCTCGGCTCA	CTGCAGCCTC	CACCTCCCAG	GTTCAAGCGA	TCCGCCTGCC	GAGTAGCTGG

[Fig.5]

2590	2600	2610	2620	2630	2640
GATTACAGGC	GCCCGCCACC	ACGCCTGGCT	AATTTTGTG	TTTGTAGAG	ATGGGGTTTC
2650	2660	2670	2680	2690	2700
GCCATGTTGG	CCAGGCTGGC	CTCGAACTGC	TCAGCTCAAG	CAATCCGCCC	GCCTCGGCCT
2710	2720	2730	2740	2750	2760
CACAAAGTCC	TAGAATTTTA	GGCATGAGCC	TCCGGGTCCG	GCCTGTGCTA	ATCCTTTCTG
2770	2780	2790	2800	2810	2820
TCCTTGGTTC	TTTATTTCCT	TTCTCTCTTT	TTCTTAGTCC	CTTTTGTTCT	TTCCCTCTCC
2830	2840	2850	2860	2870	2880
CGTTCAGTTG	GCTGTCGTTT	GAGCCTCCAC	CTTTTCACTC	CCTCCTTTCC	ACCACGATGC
2890	2900	2910	2920	2930	2940
CGAGCCCTGC	CTTGGATGGG	GACCATCAGC	GATGACCACA	ATGACCTCTC	CCTTACCAGG
2950	2960	2970	2980	2990	3000
CAGCTCCAGG	CAGTGTTTCT	GCACCGCCTT	TCCCAAGGCT	TGGGGGCTTT	TTCTAGTGGG
3010	3020	3030	3040	3050	3060
CTTTGAGCTG	CTCAATCTGG	CCTCTGCAGG	GCCGGCTCCC	AGCCCTTCCA	ACCTCCTCAC
3070	3080	3090	3100	3110	3120
AGCCCGACCT	GGGACCTAGC	CAATTCCCGG	AGAGTCTCTG	TCCCATCGTG	ACCCCTCAC
3130	3140	3150	3160	3170	3180
AACTCTCCCA	CTCACCAAAG	TCTGATGACT	GTGCTAGGGG	GTGCTTATAT	AGAGTACTGA
3190	3200	3210	3220	3230	3240
GTGTTACAAA	AGCAGAAGTC	TGGATGAGAA	CCAATTTGTG	ATATTAAGCA	GGTGGGGTGG

[Fig.6]

3250	3260	3270	3280	3290	3300
GGGTGGGGAG	TGTACCTAGG	TTCATTTTCC	CCCCTGCTTT	TCCCCTTTCC	AGTGTGTGCA
3310	3320	3330	3340	3350	3360
CTTAACCAGT	CCCTGGGCCC	TGTTCCCCAT	CCCCCTCCAA	GGCATGGATT	GGGTGGGCTT
3370	3380	3390	3400	3410	3420
GTGTGTCTTG	GGGCAGGTGG	CCCTTTCTAA	ACTCTCTGCC	TTTGCTCACC	CACAGGACAC
3430	3440	3450	3460	3470	3480
ATAGTATGAC	CATTAGGTGT	TTCGTCTCCC	ACCCATTTTC	TATGGAAAAC	CAAGGGGATC
3490	3500	3510	3520	3530	3540
GGGCCATGAT	AGCCACTGGC	AGCTT.....

【Fig.7】

